Table 1. Clinical Characteristics and Laboratory Data of All Subjects Enrolled

	Never smoker (n=2,669)	Former smoker (n=2,252)	Current smoker (n=2,157)	p value
Age, years	52.8±11.2	56.0±10.0	51.0±9.5	<0.001
Body mass index, kg/m^2	23.5±2.9	23.9±2.7	23.8±3.1	<0.001
Systolic blood pressure, mmHg	125±18	127±18	122±18	<0.001
Diastolic blood pressure, mmHg	79±11	81±11	77±11	<0.001
Antihypertensive medication (n (%))	329 (12)	403 (18)	195 (9)	<0.001
Lipid data				
Total cholesterol, mg/dL	207±32	210±31	206±33	<0.001
HDL-cholesterol, mg/dL	56±13	56±13	52±13	<0.001
LDL-cholesterol, mg/dL	128±29	129±30	126±32	0.002
Triglycerides, mg/dL	101 (73–143)	112 (80–156)	130 (90–189)	<0.001
Glucose metabolism				
Fasting glucose, mg/dL	98±16	101±19	101±24	<0.001
Hemoglobin A1c, %	5.3±0.6	5.4±0.7	5.5±0.9	<0.001
Fasting insulin, μU/mL	5.3 (3.9–7.9)	5.6 (4.0–8.4)	5.4 (3.9–8.4)	0.004
HOMA-IR	1.3 (0.9–1.9)	1.4 (1.0–2.1)	1.3 (0.9–2.1)	<0.001
Diabetes mellitus (n (%))	144 (5.4)	179 (8.0)	219 (10.2)	<0.001
Renal function				
Serum urea nitrogen, mg/dL	15.0±3.4	14.8±3.6	14.0±3.3	<0.001
Serum creatine, mg/dL	0.87±0.13	0.87±0.26	0.83±0.13	<0.001
eGFR, mL/min/1.73 m^2	69.2±9.9	69.2±9.9	73.0±10.3	<0.001
Low eGFR (n (%))	437 (16.4)	369 (16.4)	212 (9.8)	<0.001
Elevated eGFR (n (%))	60 (2.3)	45 (2.0)	99 (4.6)	<0.001
UAER, mg/g	5.2 (3.7–9.5)	6.0 (3.9–12.0)	5.7 (3.9–10.8)	<0.001
Albuminuria (n (%))	200 (7.5)	236 (1,005)	234 (10.8)	<0.001
Uric acid, mg/dL	6.1±1.2	6.2±1.2	6.2±1.3	0.019
Drinking status				
Non-drinkers (n (%))	356 (13.3)	123 (5.5)	167 (7.7)	<0.001
Former drinkers (n (%))	90 (3.4)	228 (10.1)	74 (3.4)	
Current drinkers (n (%))	2,223 (83.3)	1,901 (84.4)	1,916 (88.8)	

Data are means±SD, median (interquartile range), n, or percentage. Diabetes mellitus was diagnosed when the subject had an FPG value of ≥126 mg/dL or current use of anti-diabetic drugs. The Kruskal-Wallis test was used to evaluate differences in triglycerides, fasting insulin, HOMA-IR, and UAER among the different smoking groups. HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA-IR, homeostasis model assessment insulin resistance; eGFR, estimated glomerular filtration rate; UAER, urinary albumin excretion rate.

Methods

Study Population

Between April 2005 and August 2006, 8,054 Japanese men underwent such a screening, including the estimation of urinary albumin excretion. Among them, 2,898 were former smokers and 2,487 were current smokers. After 976 subjects were excluded for failing to complete a questionnaire about their smoking habits (the reasons for this failure were unknown), we enrolled a total of 7,078 men, including 2,252 former and 2,157 current smokers. Subjects who had quit smoking for 1 month or less and those who had quit for more than 1 month before the time of the screening were considered to be, respectively, current and former smokers. The mean age of the 8,054 individuals (that is, before exclusion) was 53.7±10.5 years, significantly higher than that of the 7,078 men selected (53.3±10.5 years, p=0.007). Therefore, there may have been some bias in selecting the study subjects; however, this was not the intention of any attending physician. In Japan, regular health check-ups for employees are a legal requirement; all or most of the costs of the screening are paid for either by the employer (accounting for about two-thirds of individuals seen at our institute) or by the subject themselves (the other third). Blood pressure was measured after about 10 min of rest by an automated sphygmomanometer. The study was approved by the Ethics Committee of the Mitsui Memorial Hospital and Faculty of Medicine, University of Tokyo.

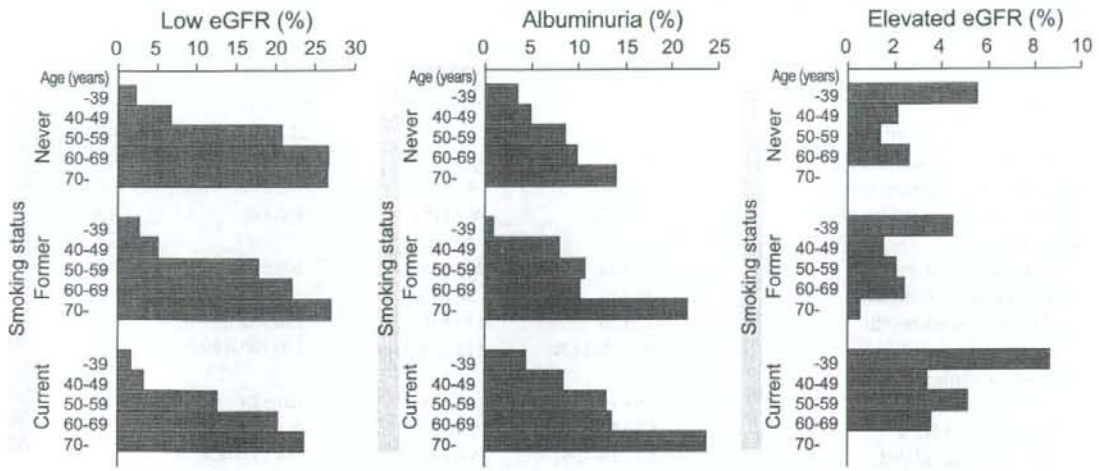

Fig. 1. *Prevalence of low eGFR, albuminuria, and elevated eGFR according to smoking status and age.*

Examination

Blood samples were taken and spot-urine specimens were obtained from the subjects in the morning after an overnight fast. Serum levels of total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), and triglycerides (TG) were determined enzymatically. Serum uric acid was measured by the uricase-peroxidase method, hemoglobin A1c was determined using the latex agglutination immunoassay, and creatinine was determined by the enzymatic method. Plasma glucose was measured by the hexokinase method, and serum insulin was measured by enzyme immunoassay. Homeostasis model assessment insulin resistance (HOMA-IR) was calculated in these individuals according to the following formula: HOMA-IR = [fasting immunoreactive insulin (μU/mL) × fasting plasma glucose (FPG; mg/dL)]/405. Creatinine and urine albumin were measured by TBA-200FR (Toshiba Medical Systems, Tochigi, Japan) and by Accute (Toshiba Medical Systems), respectively, using commercially available kits, Accuras Auto CRE (Shino-test, Tokyo, Japan) and IATRO U-ALB (TIA) (Mitsubishi Kagaku Iatron, Tokyo, Japan) respectively, according to the manufacturers' instructions. Accuracy was monitored every day by constructing X-bar and R charts using commercially available standards. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured by an automated sphygmomanometer, BP-203RVIII (Omron Colin, Tokyo, Japan). Hypertension was defined as SBP ≥140 mmHg or DBP ≥90 mmHg or current treatment with any antihypertensive drug.

Estimated GFR, Albuminuria, and Definition of CKD

Serum creatinine was calibrated by using the following formula: serum creatinine (Jaffe method) = 0.2 + serum creatinine (enzyme method). Serum creatinine was measured in mg/dL and age in years; glomerular filtration rate (GFR) was estimated by using the following equation from a simplified version of the Modification of Diet in Renal Disease (MDRD) (*9*): eGFR (mL/min/1.73 m^2) = 186.3 × (serum creatinine)$^{-1.154}$ × (age)$^{-0.203}$ × 0.881 (× 0.742 if female). In this MDRD formula, 0.881 is a coefficient for eGFR specific to the Japanese population (*10*). An eGFR of <60 mL/min/1.73 m^2 was designated as low eGFR. For the diagnosis of albuminuria, spot urine samples were collected and analyzed; albuminuria was considered present when the urinary albumin excretion ratio (UAER) expressed in mg/g creatinine, was ≥30 mg/g. Normoalbuminuria, microalbuminuria, and macroalbuminuria were defined as UAER of <30 mg/g, 30–299 mg/g, and ≥300 mg/g, respectively (*11*). Individuals were said to have CKD when they had low eGFR and/or albuminuria (*12*). Elevated eGFR was defined as an eGFR value that exceeded twice the SD of the mean eGFR value in the individuals enrolled, which was an eGFR value of >90.73 mL/min/1.73 m^2.

Statistical Analysis

Skewed variables, such as TG, fasting serum insulin, HOMA-IR, and UAER, are presented as medians (interquartile range). Other data are expressed as means±SD unless stated otherwise. Analyses of variance with Bonferroni post-hoc test, Kruskal-Wallis test, or χ^2-test were conducted as appropriate to assess the statistical significance of differences between groups. The association of smoking with CKD components (low eGFR and albuminuria) or with elevated eGFR was analyzed with a logistic regression model adjusted for all or some of the following variables: age, body mass index (BMI), SBP, FPG, HDL-C, TG, fasting serum insulin, and

current use of antihypertensive medication. Statistical analysis was performed using StatView version 5.0 (SAS Institute, Cary, USA). A value of $p<0.05$ was taken to be statistically significant.

Results

Baseline Characteristics

The mean eGFR was 70.4±10.2 mL/min/1.73 m^2, and the median UAER was 5.6 mg/g (interquartile range 3.8 to 10.6 mg/g). The mean age of former smokers was significantly higher ($p<0.001$), and that of current smokers was significantly lower ($p<0.001$), than that of never smokers (Table 1). Antidiabetic treatment was being administered to 47 (1.8%) never smokers, 85 (3.8%) former smokers, and 72 (3.3%) current smokers. The prevalence of diabetes mellitus, defined as a fasting glucose level greater than 126 mg/dL and/or taking antidiabetic medication, was significantly greater in both current and former smokers ($p<0.001$) than in never smokers. The prevalence of low eGFR was significantly lower and that of elevated eGFR was significantly higher in current than in never smokers ($p<0.001$). The prevalence of albuminuria in both former and current smokers was greater than that in never smokers ($p<0.001$).

Prevalence of Low eGFR, Albuminuria, and Elevated eGFR According to Smoking Status after Stratification by Age

As mean age differed significantly among the three groups, we plotted the prevalence of the components of CDK and elevated eGFR after stratification by age (Fig. 1). The number of individuals in the age categories of <39, 40–49, 50–59, 60–69, and ≥70 years were 7, 52, 170 155, and 53, respectively, in never smokers; 3, 24, 158, 129, and 55 in former smokers; and 4, 22, 104, 69, and 13 in current smokers. The prevalence of low eGFR and albuminuria both increased with age irrespective of smoking status.

Prevalence of Low eGFR According to Smoking Status

After adjusting for age, SBP, and FPG, logistic regression analysis revealed that current smoking showed a dose-dependent inverse association with the prevalence of low eGFR (Table 2). The prevalence of low eGFR was found to be significantly lower in current smokers who had been smoking for 10 years or longer and former smokers who had smoked for 20 years or longer. An inverse association between former smoking and low eGFR was observed in those individuals who had stopped smoking <1 year ago, but not in those who had stopped ≥1 year ago. Similar results were obtained after further adjustment for BMI, TG, HDL-C, serum insulin, and use of antihypertensive drugs; however, the association between former smoking and low eGFR was statistically significant in individuals who had stopped smoking ≥1 year ago as well as in those who had stopped <1 year ago. After adjusting for these variables, current and former smoking as a whole was associated with low eGFR, with odds ratios of 0.60 (95% confidence interval [CI] 0.50–0.73, $p<0.001$) and 0.80 (0.68–0.94, $p=0.005$), respectively.

Prevalence of Albuminuria According to Smoking Status

After adjusting for age, SBP, and FPG, logistic regression analysis showed that current smoking was statistically significantly associated with albuminuria irrespective of the amount of smoking, although the association just missed statistical significance when the amount of smoking was ≥20 cigarettes per day (Table 3). The association was also statistically significant in current smokers when the duration of smoking was ≥10 years. Former smoking tended to be associated with albuminuria when the duration of smoking was ≥10 years. Similar results were obtained after further adjustment for BMI, TG, HDL-C, serum insulin, and use of antihypertensive drugs. After adjusting for these variables, current smoking as a whole was associated with albuminuria, with an odds ratio of 1.63 (95% CI 1.34–2.03, $p<0.001$), although former smoking as a whole was not (odds ratio 1.16, 95% CI 0.94–1.44, $p=0.155$).

Prevalence of Elevated eGFR According to Smoking Status

After adjusting for age, SBP, and FPG, logistic regression analysis revealed that current smoking had a dose-dependent positive association with elevated eGFR (Table 4). In contrast, former smoking was not significantly associated with elevated eGFR irrespective of the amount or duration of smoking, or even when the cessation period was <1 year. Similar results were obtained after further adjustment for BMI, TG, HDL-C, serum insulin, and use of antihypertensive drugs. After adjusting for these variables, current smoking as a whole was associated with albuminuria with an odds ratio of 1.98 (95% CI 1.41–2.78, $p<0.001$), although former smoking as a whole was not (odds ratio 0.97, 95% CI 0.65–1.44, $p=0.878$).

Discussion

This study showed that current smoking was inversely associated with low eGFR when the amount smoked was ≥10 cigarettes per day. This association remained statistically significant after adjustment for age, SBP, and other metabolic parameters related to metabolic syndrome (BMI, HDL-C, TG, FPG, serum insulin, antihypertensive treatment) (Table 2). After adjusting for these variables, current smoking also showed a graded positive association with elevated eGFR

Table 2. Logistic Regression Analysis for Low eGFR as a Dependent Variable and Smoking Status as Independent Variables

Smoking status	Model 1		Model 2		Model 3	
	Odds ratio (95% CI)	*p*	Odds ratio (95% CI)	*p*	Odds ratio (95% CI)	*p*
Amount of smoking						
Never smoking	1.00	—	1.00	—	1.00	—
Former smoking* (cigarettes/day)						
<10	0.78 (0.55–1.11)	0.171	0.78 (0.55–1.11)	0.164	0.77 (0.54–1.09)	0.143
10–19	0.93 (0.75–1.14)	0.478	0.93 (0.76–1.14)	0.491	0.90 (0.73–1.11)	0.336
20–39	0.77 (0.61–0.96)	0.020	0.77 (0.61–0.96)	0.023	0.72 (0.57–0.91)	0.005
≥40	0.84 (0.60–1.19)	0.332	0.84 (0.60–1.19)	0.334	0.80 (0.57–1.14)	0.222
Current smoking* (cigarettes/day)						
<10	1.12 (0.81–1.54)	0.499	1.13 (0.82–1.56)	0.465	1.07 (0.77–1.48)	0.708
10–19	0.55 (0.43–0.72)	<0.001	0.56 (0.43–0.73)	<0.001	0.53 (0.40–0.69)	<0.001
20–39	0.61 (0.47–0.80)	<0.001	0.63 (0.49–0.83)	<0.001	0.55 (0.42–0.73)	<0.001
≥40	0.29 (0.12–0.72)	0.008	0.32 (0.13–0.79)	0.014	0.26 (0.10–0.65)	0.004
Duration of smoking						
Never smoking	1.00	—	1.00	—	1.00	—
Former smoking* (years)						
<5	0.71 (0.42–1.20)	0.200	0.70 (0.42–1.19)	0.189	0.72 (0.42–1.21)	0.213
5–9	0.87 (0.63–1.20)	0.383	0.86 (0.63–1.19)	0.371	0.82 (0.59–1.14)	0.232
10–19	1.00 (0.79–1.25)	0.965	0.99 (0.79–1.25)	0.947	0.97 (0.77–1.22)	0.789
≥20	0.76 (0.62–0.93)	0.007	0.77 (0.63–0.94)	0.010	0.72 (0.59–0.89)	0.002
Current smoking* (years)						
<5	0.94 (0.31–2.79)	0.904	1.00 (0.33–2.99)	0.999	1.12 (0.37–3.37)	0.837
5–9	1.11 (0.48–2.56)	0.805	1.15 (0.50–2.64)	0.750	1.18 (0.51–2.71)	0.701
10–19	0.53 (0.32–0.89)	0.017	0.54 (0.32–0.91)	0.021	0.50 (0.30–0.84)	0.009
≥20	0.64 (0.53–0.78)	<0.001	0.66 (0.55–0.80)	<0.001	0.60 (0.49–0.73)	<0.001
Years of cessation						
Never smoking	1.00	—	1.00	—	1.00	—
Former smoking*						
Last smoked <1 year ago	0.51 (0.29–0.88)	0.015	0.52 (0.30–0.89)	0.018	0.51 (0.29–0.88)	0.015
Last smoked ≥1 year ago	0.87 (0.74–1.02)	0.079	0.87 (0.74–1.02)	0.081	0.83 (0.71–0.98)	0.025

Model 1, adjusted for age; model 2, age, SBP, and FPG; model 3, age, SBP, FPG, BMI, HDL-C, TG, fasting serum insulin, and current use of antihypertensive drug. *Never smoking was used as reference. eGFR, estimated glomerular filtration rate; CI, confidence interval; SBP, systolic blood pressure; FPG, fasting blood glucose; BMI, body mass index; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; TG, triglyceride.

(Table 4). On the other hand, former smoking showed a statistically significant inverse association with low eGFR, whereas the association with either albuminuria or elevated eGFR did not reach statistical significance irrespective of the duration of smoking (Tables 3, 4). Interestingly, the association between cigarette smoking and elevated eGFR lost statistical significance even within 1 year after quitting (Table 4). These findings collectively suggest that cigarette smoking decreases the prevalence of low eGFR and increases the prevalence of albuminuria and elevated eGFR—an association that is markedly weakened (for low eGFR and albuminuria) or abolished (for elevated eGFR) after quitting smoking.

Several studies have demonstrated a positive association between smoking and albuminuria, some of which showed statistically significant associations in both current and former smokers (*13*), while others demonstrated only in current smokers (*5*, *6*). In the current study, after adjusting for age and variables related to metabolic syndrome, current smoking as a whole showed a statistically significant inverse association with low eGFR and positive ones with albuminuria and elevated eGFR. On the other hand, former smoking, as a whole, showed a statistically significant inverse association with low eGFR, but the association between former smoking and albuminuria was not significant. These findings are in agreement with the results of the third National Health and Nutrition Examination Survey, which showed that the cessation of smoking weakens or abolishes the increase in albuminuria (*5*).

Table 3. Logistic Regression Analysis for Albuminuria as a Dependent Variable and Smoking Status as Independent Variables

Smoking status	Model 1		Model 2		Model 3	
	Odds ratio (95% CI)	*p*	Odds ratio (95% CI)	*p*	Odds ratio (95% CI)	*p*
Amount of smoking						
Never smoking	1.00	—	1.00	—	1.00	—
Former smoking* (cigarettes/day)						
<10	0.65 (0.39–1.11)	0.112	0.73 (0.42–1.24)	0.239	0.70 (0.41–1.21)	0.203
10–19	1.49 (1.16–1.91)	0.002	1.46 (1.12–1.90)	0.005	1.45 (1.12–1.89)	0.006
20–39	1.26 (0.96–1.65)	0.102	1.08 (0.81–1.44)	0.601	1.03 (0.77–1.37)	0.857
≥40	1.56 (1.05–2.33)	0.029	1.27 (0.84–1.92)	0.261	1.16 (0.77–1.77)	0.482
Current smoking* (cigarettes/day)						
<10	1.57 (1.08–2.30)	0.019	1.72 (1.15–2.56)	0.008	1.67 (1.12–2.51)	0.012
10–19	1.61 (1.24–2.08)	<0.001	1.64 (0.25–2.16)	<0.001	1.70 (1.28–2.24)	<0.001
20–39	1.66 (1.27–2.18)	<0.001	1.56 (1.17–2.08)	0.002	1.53 (1.14–2.06)	0.005
≥40	2.43 (1.37–4.32)	0.003	1.88 (0.99–3.55)	0.053	1.81 (0.96–3.41)	0.068
Duration of smoking						
Never smoking	1.00	—	1.00	—	1.00	—
Former smoking* (years)						
<5	0.52 (0.23–1.20)	0.124	0.57 (0.25–1.31)	0.184	0.55 (0.24–1.29)	0.169
5–9	0.69 (0.43–1.13)	0.137	0.72 (0.44–1.18)	0.193	0.70 (0.42–1.15)	0.159
10–19	1.57 (1.19–2.06)	0.001	1.36 (1.02–1.81)	0.035	1.33 (1.00–1.77)	0.050
≥20	1.45 (1.14–1.84)	0.003	1.33 (1.04–1.71)	0.025	1.27 (0.99–1.64)	0.060
Current smoking* (years)						
<5	1.53 (0.46–5.14)	0.491	1.03 (0.24–4.52)	0.965	1.19 (0.28–5.04)	0.809
5–9	0.88 (0.27–2.88)	0.838	0.84 (0.25–2.81)	0.773	0.89 (0.27–2.96)	0.843
10–19	1.83 (1.21–2.78)	0.004	1.82 (1.17–2.83)	0.008	1.73 (1.10–2.72)	0.017
≥20	1.66 (1.35–2.04)	<0.001	1.64 (1.32–2.05)	<0.001	1.66 (1.32–2.08)	<0.001
Years of cessation						
Never smoking	1.00	—	1.00	—	1.00	—
Former smoking*						
Last smoked <1 year ago	1.41 (0.83–2.37)	0.204	1.28 (0.74–2.22)	0.374	1.28 (0.74–2.22)	0.382
Last smoked ≥1 year ago	1.29 (1.05–1.58)	0.014	1.20 (0.97–1.48)	0.097	1.16 (0.93–1.43)	0.184

Models as in Table 2. *Never smoking was used as reference. CI, confidence interval.

We also showed here that current smoking dose-dependently reduced the prevalence of low eGFR. In agreement with our result, some studies have shown that current smoking is associated with higher creatinine clearance or GFR in the general population (*14*) and in type 2 diabetic patients (*7*). On the other hand, however, some other studies have shown that current smoking decreases GFR in community-dwelling subjects (*8*) and type 2 diabetic patients (*15*). What causes these conflicting results has not been fully clarified; however, insulin resistance, which might be enhanced by smoking (*16*), may have a role in these discrepant observations, as it may lead to a decrease (*17*) or an elevation (*18*) of eGFR.

In the current study, current smoking, but not former smoking, was dose-dependently positively associated with elevated eGFR (Table 2). Ekberg *et al.* reported that glomerular hyperfiltration was more prevalent in smokers than in nonsmokers (*19*). In addition, in a substudy of the PREVEND study (Prevention of Renal and Vascular End-stage Disease), Pinto-Sietsma *et al.* reported that current smoking showed a dose-dependent association with elevated eGFR in nondiabetic subjects, which disappeared after smoking ceased (*13*). We cannot conclude the mechanism by which smoking elevates GFR in the Japanese population from this type of cross-sectional study; however, it is possible that pre-glomerular vessels and glomerular obsolescence lead to hypertrophy and hyperfiltration of remnant glomeruli after repeated transient decreases in renal plasma flow and GFR induced by smoking, which eventually result in elevated GFR (*14*, *20*). It is recognized that glomerular hyperfiltration is not a rare occurrence in individuals with impaired glucose metabolism (*21*, *22*) or even in apparently healthy young men (*23*). It should be noted that glomerular hyperfiltration represents a new marker of clustering of metabolic risk factors even before overt features of cardiovascular disease are manifest (*23*). Thus, the increase

Table 4. Logistic Regression Analysis for Elevated eGFR as a Dependent Variable and Smoking Status as Independent Variables

Smoking status	Model 1		Model 2		Model 3	
	Odds ratio (95% CI)	*p*	Odds ratio (95% CI)	*p*	Odds ratio (95% CI)	*p*
Amount of smoking						
Never smoking	1.00	—	1.00	—	1.00	—
Former smoking* (cigarettes/day)						
<10	0.57 (0.20–1.57)	0.276	0.61 (0.22–1.70)	0.348	0.61 (0.22–1.70)	0.342
10–19	0.81 (0.46–1.44)	0.477	0.77 (0.43–1.38)	0.379	0.78 (0.45–1.40)	0.405
20–39	1.40 (0.93–2.37)	0.207	1.21 (0.71–2.06)	0.477	1.32 (0.77–2.25)	0.310
≥40	1.50 (0.63–3.57)	0.358	1.24 (0.53–3.05)	0.592	1.41 (0.58–3.41)	0.447
Current smoking* (cigarettes/day)						
<10	0.72 (0.31–1.67)	0.438	0.70 (0.30–1.63)	0.403	0.71 (0.30–1.69)	0.440
10–19	2.03 (1.37–3.01)	<0.001	1.87 (1.25–2.81)	0.002	1.93 (1.28–2.90)	0.002
20–39	2.50 (1.67–3.75)	<0.001	2.35 (1.56–3.54)	<0.001	2.56 (1.68–3.91)	<0.001
≥40	3.10 (1.30–7.39)	0.011	2.46 (1.01–6.00)	0.049	2.81 (1.13–6.99)	0.026
Duration of smoking						
Never smoking	1.00	—	1.00	—	1.00	—
Former smoking* (years)						
<5	0.95 (0.34–2.66)	0.924	1.02 (0.37–2.87)	0.965	1.01 (0.36–2.83)	0.986
5–9	0.48 (0.17–1.32)	0.154	0.50 (0.18–1.38)	0.181	0.50 (0.18–1.39)	0.183
10–19	1.01 (0.57–1.80)	0.963	0.91 (0.51–1.64)	0.762	0.95 (0.53–1.70)	0.853
≥20	1.36 (0.80–2.29)	0.254	1.18 (0.69–2.00)	0.548	1.28 (0.75–2.19)	0.365
Current smoking* (years)						
<5	2.54 (0.59–11.04)	0.214	2.03 (0.43–0.96)	0.369	1.89 (0.38–9.45)	0.439
5–9	2.06 (0.71–5.97)	0.186	2.05 (0.71–5.96)	0.188	1.97 (0.67–5.77)	0.216
10–19	1.70 (0.99–2.92)	0.053	1.59 (0.92–2.74)	0.097	1.68 (0.97–2.92)	0.065
≥20	2.10 (1.48–2.98)	<0.001	1.94 (1.36–2.78)	<0.001	2.04 (1.42–2.95)	<0.001
Years of cessation						
Never smoking	1.00	—	1.00	—	1.00	—
Former smoking*						
Last smoked <1 year ago	1.49 (0.63–3.50)	0.364	1.32 (0.55–3.15)	0.534	1.37 (0.57–3.29)	0.479
Last smoked ≥1 year ago	0.97 (0.64–1.47)	0.871	0.90 (0.59–1.37)	0.628	0.94 (0.62–1.43)	0.768

Abbreviations and models as in Table 2. *Never smoking was used as reference. CI, confidence interval.

in eGFR caused by cigarette smoking may not simply be a preferable or an innocuous observation. In addition, current smoking may increase the prevalence of both low GFR and elevated GFR in the same population (*13*, *15*), which suggests the possibility that a simple comparison of the eGFR between smokers and nonsmokers may lead to an inappropriate conclusion.

Our study has some limitations. First, we used the MDRD formula with the Japanese coefficient of 0.881 for the estimation of GFR (*10*), and a recent study has shown that this formula may underestimate GFR in the inulin clearance range of over 60 mL/min/1.73 m^2 in the Japanese population. Second, in the MDRD formula used, muscle mass was not taken into consideration for GFR estimation. Because the serum creatinine value is the balance of the release from skeletal muscle and removal by the kidneys, both muscle mass and renal function are important determinants. Recent studies have shown that anthropometric/demographic variables, such as age, gender, height, and weight, may not adequately account for variance in muscle mass, and that measures of muscle mass, which can be clinically obtainable (*24*), may improve the estimation of GFR (*25*). Third, owing to the cross-sectional nature of the current study, we cannot determine whether or not elevation of eGFR in smokers modulates long-term renal prognosis.

In conclusion, by analyzing the cross-sectional data of Japanese men who underwent a general health screening, we showed that current smoking was dose-dependently associated inversely with low eGFR and positively with albuminuria and elevated eGFR—associations that were weakened or abolished after quitting. We may need to take into account an individual's smoking status when assessing the eGFR and

thus the presence of CKD, especially when urine data are not available, as smoking may increase the prevalence of not only albuminuria but also hyperfiltration. Whether or not elevation of eGFR owing to cigarette smoking acts protectively for renal function in the long term, and whether or not elevation of eGFR by current smoking is an acute and transient phenomenon that does not modulate long-term renal prognosis, need to be investigated in future longitudinal studies.

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Relationship between Albuminuria, Low eGFR, and Carotid Atherosclerosis in Japanese Women

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Key Words
Chronic kidney disease · Carotid atherosclerosis · Carotid atherosclerosis, women · Health screening

Abstract
In this cross-sectional study, we have investigated whether chronic kidney disease components were associated with carotid plaque and carotid intima-media thickening in women. Between April 2005 and May 2006, 830 women underwent general health screening including carotid ultrasonography and urinary albumin excretion, and were enrolled in the study. Of these individuals examined, 83 (10%) had albuminuria, 203 (24%) had low estimated GFR (eGFR), and 24 (3%) had both albuminuria and low eGFR. Univariate analysis showed that albuminuria, but not low eGFR, was associated with carotid plaque, and that both albuminuria and low eGFR were positively associated with carotid intima-media thickening. Age-adjusted logistic regression analysis showed that albuminuria was positively associated with carotid plaque with an odds ratio of 2.48 (95% CI 1.49–4.11, $p < 0.001$). On the other hand, association between albuminuria and carotid intima-media thickening was not statistically significant after age adjustment. Positive association between albuminuria and carotid plaque was present when either hypertension or high fasting glucose was absent. In conclusion, in Japanese women who underwent general health screening, albuminuria, but not low eGFR, was positively associated with carotid plaque.

Introduction

Increasing evidence indicates that an early stage of chronic kidney disease (CKD), reflected by either reduced glomerular filtration rate (GFR) and albuminuria/proteinuria, may increase the incidence of not only end-stage renal disease, but also ischemic cardiac and cerebrovascular diseases [1, 2]. In addition, it has been reported that prevalence carotid artery intima-media thickening and carotid plaque, both of which are risk factors for stroke [3] and coronary artery disease [4, 5], are more common in subjects with CKD than those without [6, 7]. We previously reported that both albuminuria and low estimated GFR (eGFR) were a risk factor for carotid intima-media thickening in Japanese men. In the present study, we have addressed the relationship between CKD components and carotid atherosclerosis in Japanese women.

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Subjects and Methods

Study Population

Between April 2005 and May 2006, 830 women underwent general health screening (Ningen Dock) including carotid ultrasonography as a part of the health screening course, and were enrolled in the present study. The study was approved by The Ethical Committee of Mitsui Memorial Hospital and University of Tokyo, Faculty of Medicine.

Laboratory Analysis

Blood samples were taken from the subjects after an overnight fast. Serum levels of total cholesterol, HDL-cholesterol, and triglycerides were determined enzymatically. Serum uric acid was measured by the uricase-peroxidase method, hemoglobin A_{1C} was determined using the latex agglutination immunoassay, and creatinine was determined by the enzymatic method. Plasma glucose was measured by the hexokinase method and serum insulin was measured by enzyme immunoassay. Homeostasis model assessment insulin resistance (HOMA-IR) was calculated in these individuals according to the following formula: HOMA-IR = [fasting immunoreactive insulin (μU/ml) × fasting plasma glucose (FPG; mg/dl)]/405.

Creatinine and urine albumin were measured by TBA-200FR (Toshiba Medical Systems, Tochigi, Japan) and by Accute (Toshiba Medical Systems), respectively, using commercially available kits, Accuras Auto CRE (Shino-test, Tokyo, Japan) and IATRO U-ALB (TIA) (Mitsubishi Kagaku Iatron, Tokyo, Japan), respectively. Serum creatinine was calibrated using the following formula: serum creatinine (Jaffe method) = 0.2 + serum creatinine (enzyme method). GFR was estimated by equations of a simplified version of Modification of Diet in Renal Disease [8], where 0.881 is a coefficient for eGFR specific to the Japanese population [9], $eGFR = 186.3 \times (\text{serum creatinine})^{-1.154} \times (\text{age})^{-0.203} \times 0.881 \times 0.742$ (for female). Individuals were classified as having low eGFR when their eGFR values were <60 ml/min/1.73 m^2 [10]. For the diagnosis of albuminuria, spot urine samples were collected and analyzed; albuminuria was defined to be present when the urinary albumin excretion ratio (UAER), expressed as milligrams as per gram creatinine, was ≥30 mg/g. Normo-, micro-, and macroalbuminuria were defined as an UAER of <30, 30–299, and 300 mg/g or more, respectively. Albuminuria and low eGFR are the components of CKD [10].

Carotid Ultrasonography

Carotid artery status was studied and analyzed as described previously [11]. In brief, carotid artery status was assessed by high-resolution B-mode ultrasonography, using a machine (Sonolayer SSA270A, Toshiba, Japan) equipped with a 7.5-MHz transducer (PLF-703ST, Toshiba). The carotid arteries were examined bilaterally at the levels of the common carotid, the bifurcation, and the internal carotid arteries from transverse and longitudinal orientations by trained sonographers. Carotid intima-media wall thickening was said to occur when the intima-media thickness which was measured at the far wall of the distal 10 mm of the common carotid artery was ≥1.0 mm. Carotid plaque was defined when there is portion that shows the thickness of intima-media complex ≥1.1 mm [12] with the focal protrusion or point(s) of inflexion.

Statistical Analysis

Skewed variables (triglycerides, HOMA-IR, UAER) are presented as median (interquartile range). Other data are expressed as the mean ± SD unless stated otherwise. Analyses of variance, the Mann-Whitney U test, χ^2 tests, and logistic regression analysis were conducted as appropriate to assess the statistical significance of differences between groups using computer software, StatView (Version 5.0; SAS Institute, Cary, N.C., USA) and Dr. SPSS II (Chicago, Ill., USA). A value of $p < 0.05$ was taken to be statistically significant.

Results

Baseline Characteristics

The mean age ± SD of the individuals enrolled was 57.3 ± 11.0 years. Of the 830 individuals examined, 83 (10.0%) had albuminuria, 203 (24.5%) had low eGFR, and 24 (2.9%) had both albuminuria and low eGFR. Therefore, 262 (31.6%) subjects were said to have CKD in our study population. Among 83 who had albuminuria, 75 (9.0%) had microalbuminuria and the remaining 8 (1.0%) had macroalbuminuria. Prevalence of low eGFR in individuals who did not have albuminuria [179/747 (24.0%)] and that in those who had albuminuria [24/83 (28.9%)] did not significantly differ ($p = 0.389$, by the χ^2 test). Individuals with albuminuria had a greater HOMA-IR value than those without CKD (table 1). After adjusting for age and smoking status, logistic regression analysis showed that the odds ratios of albuminuria and low eGFR for increased insulin resistance (defined here as HOMA-IR of ≥2.0) was 4.17 (95% CI 2.22–7.83, $p < 0.001$) and 0.86 (95% CI 0.53–1.40, $p = 0.543$), respectively. When only 630 individuals who had an FPG level of <126 mg/dl and were not taking antidiabetic medication were analyzed, association between albuminuria and increased insulin resistance was still significant with an odds ratio of 2.63 (95% CI 1.49–4.69, $p < 0.001$).

Association between CKD Components and Carotid Plaque

Carotid plaque was more frequently found in individuals with albuminuria than in non-CKD subjects (fig. 1). On the other hand, prevalence of carotid plaque was not significantly different between individuals with low eGFR and those without CKD. Logistic regression analysis showed that, as compared with the no-CKD group, albuminuria was associated with a higher prevalence of carotid plaque after adjusting for age only and after adjustment for age, systolic blood pressure (SBP), FPG, and smoking status (table 2). When HOMA-IR was added as

Table 1. Clinical characteristic and laboratory data in subjects with and without albuminuria and/or low eGFR

	No CKD (n = 568)	CKD (n = 262)	p value	Albuminuria (n = 83)	Low eGFR (n = 203)
Age, years	54.5 ± 10.4	63.4 ± 9.8	<0.001	62.3 ± 11.2	64.1 ± 8.9
Body mass index	21.4 ± 3.0	22.0 ± 3.4	0.016	22.3 ± 4.4	21.8 ± 2.8
Systolic blood pressure, mm Hg	119 ± 19	125 ± 21	<0.001	136 ± 23	122 ± 20
Diastolic blood pressure, mm Hg	74 ± 12	77 ± 13	0.006	82 ± 14	75 ± 12
Antihypertensive medication, n (%)	43 (7.6)	41 (15.6)	<0.001	25 (30.1)	24 (11.8)
Antidiabetic medication, n (%)	4 (0.7)	2 (0.7)	>0.999	1 (1.2)	2 (1.0)
Laboratory data					
Serum calcium, mg/dl	9.3 ± 0.3	9.4 ± 0.3	<0.001	9.4 ± 0.4	9.4 ± 0.3
Serum phosphate, mg/dl	3.7 ± 0.4	3.7 ± 0.4	0.850	3.7 ± 0.4	3.8 ± 0.4
Serum albumin, g/dl	4.5 ± 0.2	4.5 ± 0.2	0.162	4.5 ± 0.2	4.5 ± 0.2
Serum urea nitrogen, mg/dl	13.7 ± 3.3	15.8 ± 4.0	<0.001	15.9 ± 5.4	16.4 ± 4.0
Serum creatinine, mg/dl	0.6 ± 0.1	0.7 ± 0.2	<0.001	0.7 ± 0.3	0.8 ± 0.2
eGFR, ml/min/1.73 m^2	71 ± 8	59 ± 10	<0.001	67 ± 15	55 ± 6
UAER, mg/g	8.0 (5.2–13.1)	11.7 (6.5–37.5)	<0.001	55.8 (39.1–101.2)	8.7 (5.5–15.0)
Uric acid, mg/dl	4.5 ± 0.9	5.1 ± 1.1	<0.001	5.0 ± 1.3	5.2 ± 1.1
Total cholesterol, mg/dl	219 ± 36	230 ± 34	<0.001	227 ± 41	232 ± 32
HDL cholesterol, mg/dl	68 ± 15	67 ± 16	0.841	66 ± 16	68 ± 16
Triglycerides, mg/dl	75 (56–104)	86 (65–119)	<0.001	87 (64–120)	86 (65–119)
Fasting glucose, mg/dl	92 ± 13	96 ± 23	<0.001	105 ± 36	93 ± 11
Hemoglobin A_{1C}, %	5.2 ± 0.5	5.4 ± 0.7	<0.001	5.5 ± 1.0	5.3 ± 0.5
Carotid ultrasonography					
IMT, mm	0.67 ± 0.17	0.75 ± 0.19	<0.001	0.76 ± 0.21	0.75 ± 0.20
max. IMT, mm	1.19 ± 0.54	1.31 ± 0.56	0.003	1.40 ± 0.58	1.27 ± 0.54
Plaque, n (%)	226 (40)	129 (49)	0.013	56 (67)	89 (44)
HOMA-IR	1.04 (0.74–1.47)	1.16 (0.81–1.80)	0.004	1.44 (0.85–2.35)	1.13 (0.81–1.62)
Smoking status					
Never, n (%)	490 (86.3)	234 (89.3)	0.122	71 (85.5)	186 (91.6)
Former, n (%)	34 (6.0)	7 (2.7)		3 (3.6)	5 (2.5)
Current, n (%)	44 (7.7)	21 (8.0)		9 (10.8)	12 (5.9)

Data are means ± SD, median (interquartile range), n, or percentage. UAER indicates the urinary albumin excretion rate. The Mann-Whitney U test was used to evaluate differences in triglycerides, fasting insulin, HOMA-IR, and UAER between no-CKD and CKD groups.

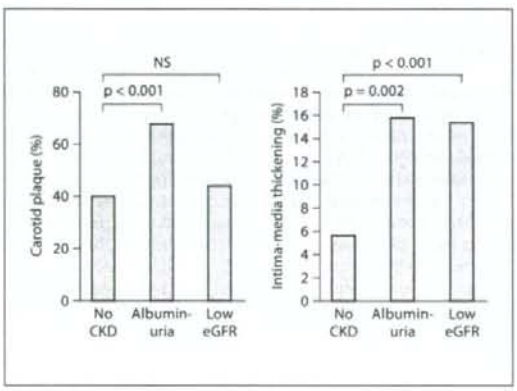

Fig. 1. Prevalence of carotid plaque and carotid artery intima-media thickening and in individuals with or without CKD components.

Table 2. Logistic regression analysis for carotid plaque as a dependent variable

	Odds ratio (95% CI) of albuminuria	p value	Odds ratio (95% CI) of low eGFR	p value
Whole				
Unadjusted	3.11 (1.92–5.03)	<0.001	1.06 (0.77–1.46)	0.723
Adjusted for age	2.48 (1.49–4.11)	<0.001	0.54 (0.37–0.78)	0.001
Adjusted for age, SBP, FPG, and smoking status	2.13 (1.26–3.61)	0.005	0.56 (0.39–0.81)	0.002
Subjects with hypertension (n = 204)				
Unadjusted	1.88 (0.93–3.80)	0.079	0.67 (0.36–1.26)	0.216
Adjusted for age	1.65 (0.80–3.39)	0.173	0.45 (0.22–0.90)	0.025
Adjusted for age, SBP, FPG, and smoking status	1.69 (0.78–3.65)	0.182	0.45 (0.22–0.92)	0.028
Subjects without hypertension (n = 626)				
Unadjusted	3.39 (1.70–6.76)	<0.001	1.23 (0.85–1.78)	0.282
Adjusted for age	3.08 (1.48–6.42)	0.003	0.60 (0.39–0.93)	0.021
Adjusted for age, SBP, FPG, and smoking status	2.83 (1.34–5.96)	0.006	0.61 (0.40–0.95)	0.027
Subjects with high fasting glucose (n = 57)				
Unadjusted	1.36 (0.42–4.39)	0.613	0.87 (0.26–2.96)	0.826
Adjusted for age	1.23 (0.36–4.25)	0.742	0.52 (0.13–2.05)	0.353
Adjusted for age, SBP, and smoking status	1.30 (0.26–6.58)	0.751	0.46 (0.11–1.87)	0.275
Subjects without high fasting glucose (n = 773)				
Unadjusted	3.35 (1.95–5.74)	<0.001	1.08 (0.77–1.50)	0.669
Adjusted for age	2.68 (1.52–4.73)	<0.001	0.55 (0.37–0.80)	0.002
Adjusted for age, SBP, and smoking status	2.39 (1.34–4.24)	0.003	0.57 (0.38–0.82)	0.003

an additional covariate to this statistical mode, albuminuria was still associated with carotid plaque with an odds ratio of 2.12 (95% CI 1.25–3.60, p = 0.005). Positive association between albuminuria and carotid plaque was statistically significant also in individuals without hypertension, defined here as SBP of ≥140 mm Hg, diastolic blood pressure of ≥90 mm Hg or taking antihypertensive medication, and was also statistically significant in those without high fasting glucose, defined here as an FPG level of ≥110 mg/dl or taking antidiabetic medication (table 2). By contrast, low eGFR was associated with a lower prevalence of carotid plaque after adjusting for age or after adjustment for age, SBP, FPG, and smoking status.

Association between CKD Components and Carotid Intima-Media Thickening

Carotid intima-media thickening was more frequently found in individuals with albuminuria or in those with low eGFR than non-CKD subjects (fig. 1). After adjusting for age, association between either of these two CKD components and intima-media thickening lost statistical significance, irrespective of the status of hypertension or high fasting glucose (table 3).

We also analyzed the database which contained 3,318 women who underwent general health screening between 2003 and 2007; not all of them had data of urinary albumin excretion. Age-adjusted logistic regression analysis showed that odds ratio of low eGFR for carotid plaque was 0.67 (95% CI 0.56–0.82, $p < 0.001$) and that for carotid intima-media thickening was 1.11 (95% CI 0.80–1.54, p = 0.54). Therefore, the inverse association between low eGFR and carotid plaque and no significant association between low eGFR and carotid intima-media thickening were observed also in the database containing a female population of larger size.

In addition, after adding the data of men (n = 1,705), we investigated the interaction between gender and CKD components. When carotid plaque was used as a dependent variable, gender showed a significant interaction with both albuminuria and low eGFR ($p < 0.05$). On the other hand, when carotid intima-media thickening was used as a dependent variable, gender showed a significant interaction with albuminuria ($p < 0.05$), but not with low eGFR (p = 0.83).

Serum Levels of Calcium and Phosphorus and Carotid Atherosclerosis in CKD Subjects

We then investigated whether serum levels of calcium and phosphorus were associated with carotid atherosclerosis in individuals with CKD. In individuals with CKD, age-adjusted logistic regression analysis showed that an

Table 3. Logistic regression analysis for carotid intima-media thickening as a dependent variable

	Odds ratio (95% CI) of albuminuria	p value	Odds ratio (95% CI) of low eGFR	p value
Whole				
Unadjusted	2.21 (1.15–4.23)	0.017	2.64 (1.61–4.36)	<0.001
Adjusted for age	1.26 (0.61–2.61)	0.534	1.25 (0.73–2.15)	0.416
Adjusted for age, SBP, FPG, and smoking status	0.74 (0.33–1.66)	0.469	1.34 (0.77–2.33)	0.304
Subjects with hypertension (n = 204)				
Unadjusted	1.28 (0.55–2.97)	0.079	2.68 (1.25–5.74)	0.011
Adjusted for age	0.93 (0.38–2.30)	0.882	1.81 (0.81–4.05)	0.148
Adjusted for age, SBP, FPG, and smoking status	0.89 (0.34–2.32)	0.806	1.82 (0.81–4.11)	0.149
Subjects without hypertension (n = 626)				
Unadjusted	2.04 (0.68–6.11)	0.201	2.70 (1.36–5.35)	0.005
Adjusted for age	1.17 (0.32–4.26)	0.816	1.04 (0.49–2.21)	0.915
Adjusted for age, SBP, FPG, and smoking status	0.69 (0.16–2.97)	0.621	1.09 (0.51–2.36)	0.822
Subjects with high fasting glucose (n = 57)				
Unadjusted	0.53 (0.10–2.82)	0.460	1.40 (0.31–6.36)	0.661
Adjusted for age	0.39 (0.06–2.33)	0.300	0.84 (0.17–4.32)	0.838
Adjusted for age, SBP, and smoking status	0.33 (0.04–2.76)	0.304	0.76 (0.15–3.91)	0.743
Subjects without high fasting glucose (n = 773)				
Unadjusted	2.63 (1.29–5.34)	0.008	2.90 (1.70–4.95)	<0.001
Adjusted for age	1.47 (0.66–3.28)	0.350	1.47 (0.66–3.28)	0.350
Adjusted for age, SBP, and smoking status	1.10 (0.48–2.54)	0.280	1.39 (0.77–2.50)	0.280

odds ratio of serum calcium (per 1 mg/dl increase) was 1.18 (95% CI 0.54–2.57, p = 0.680) for carotid plaque, and 1.51 (95% CI 0.48–4.73, p = 0.477) for carotid intima-media thickening. In these individuals, age-adjusted logistic regression analysis showed that an odds ratio of serum phosphorus (per 1 mg/dl increase) was 0.79 (95% CI 0.42–1.51, p = 0.476) for carotid plaque, and 0.41 (95% CI 0.16–1.06, p = 0.066) for carotid intima-media thickening.

Discussion

In the current study, we have investigated the association between components of CKD (low eGFR and albuminuria) and carotid atherosclerosis in women who underwent general health screening. Univariate analysis showed that albuminuria, but not low eGFR, was significantly positively associated with carotid plaque. The association between albuminuria and carotid plaque remained statistically significant after adjustment for age, SBP, FPG, and smoking status. In addition, positive association between albuminuria and carotid plaque was observed in women without hypertension or in those without high fasting glucose. Although both albuminuria and low eGFR was associated with carotid intima-media thickening by univariate analysis, statistical significance was lost after multivariate adjustment, irrespective of the status of hypertension or high fasting glucose.

Evidence is accumulating that presence of early-phase renal disease may increase the risk of atherosclerotic diseases; however, information over the possible relationship between CKD, especially low eGFR, and carotid artery atherosclerosis seems to be limited. Zhang et al. [6] analyzed the data of 1,264 invited Chinese residents (543 men, 721 women) aged 40 years or older. They found that GFR was negatively associated with carotid intima-media thickness in univariate analysis; however, the observed association lost its statistical significance after adjusting for other atherogenic risk factors. Briet et al. [2] reported that carotid intima-media thickness did not differ significantly between individuals with decreased eGFR and those without. In the previous paper, we reported that low eGFR was associated with carotid intima-media thickening in male individuals when individuals had other atherogenic risk factors, such as hypertension, impaired glucose metabolism, or cigarette smoking [13]. Together with these previous observations, findings in the current study suggested that risk factor properties of low eGFR on carotid atherosclerosis might be different according to the gender. Low eGFR was found to be, although unexpectedly, inversely associated with carotid

plaque in the current study; however, whether there is truly such a relationship or not needs to be further addressed in future studies after increasing the number of enrolled subjects.

Compared to low eGFR, the association of albuminuria with carotid atherosclerosis has more consistently demonstrated by cross-sectional and prospective studies [14–16]. It is possible that impaired glucose/lipid metabolism may represent a mechanism underlying this observed link because albuminuria is known to be associated metabolic syndrome and increased insulin resistance. Consistent with this idea, in the current study, albuminuria was associated with increased insulin resistance (HOMA-IR of ≥2.0) with an odds ratio of 4.17. It is of note, however, that association between albuminuria and carotid plaque was found to be independent of SBP, FPG, and HOMA-IR.

It has been reported that serum phosphorus concentration was associated with coronary arterial wall calcification and carotid intima-media thickening in patients with end-stage renal disease [17, 18]. Therefore, here we examined whether serum phosphorus levels were associated with carotid atherosclerosis in individuals with CKD. Serum phosphorus levels were not significantly different between CKD-positive and -negative individuals (table 1). Serum phosphorus levels were found not to be significantly associated with carotid plaque or intima-media thickening by age-adjusted logistic regression analysis, which was in agreement with the observation of Maeda et al. [19]. On the other hand, we previously showed both serum calcium and phosphorus were associated with carotid plaque by analyzing 5,732 subjects [20]. Therefore, the findings in the current study should be re-evaluated after increasing the number of individuals enrolled.

There are several study limitations in the current study. We used the Modification of Diet in Renal Disease formula with the Japanese coefficient of 0.881 for the estimation of GFR [9], which may not be very accurate for values >60 ml/min/1.73 m^2. We could not find a significant association between serum levels of calcium/phosphorus and carotid atherosclerosis in individuals with CKD. It is possible that there may be closer association between these variables when eGFR is much lower. In the current study population, however, only a small fraction of subjects (n = 3) had eGFR level <30 ml/min/1.73 m^2.

In conclusion, by analyzing cross-sectional data from Japanese women who underwent general health screening, we found that albuminuria, but not low eGFR, was positively associated with carotid plaque in univariate analysis. Association between albuminuria and carotid plaque remained statistically significant after multivariate adjustment. It was found to be significant also in individuals without hypertension or in those without high fasting glucose. It may be proposed that, when assessing a possible association between CKD and carotid atherosclerosis, albuminuria and low eGFR should be analyzed separately in women undergoing general health screening, or Ningen Dock.

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INTERNAL MEDICINE

CASE REPORT

Combined Treatment with Oral Kanamycin and Parenteral Antibiotics for a Case of Persistent Bacteremia and Intestinal Carriage with *Campylobacter coli*

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Abstract

Campylobacter coli (*C. coli*) is a rare pathogen of bacteremia, but in immunocompromised hosts, *C. coli* occasionally causes bacteremia which can be refractory to antibiotic treatment. We report a case of *C. coli* bacteremia in a patient with X-linked agammaglobulinemia. Bacteremia relapsed repeatedly in spite of treatment with combined intravenous antibiotics. *C. coli* was observed in the biopsy specimens from the intestinal mucosa, suggesting intestinal carriage and reservoir of recurring infection. The addition of oral kamamycin with intravenous antibiotics was successful in eradicating *C. coli* from the blood and intestine.

Key words: *Campylobacter coli*, X-linked agammaglobulinemia, oral kamamycin

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Introduction

Campylobacter jejuni and *Campylobacter coli* (*C. coli*) frequently cause enteritis, but rarely cause bacteremia or extraintestinal infections in immunologically normal hosts (1, 2). However, in immunocompromised hosts, especially in patients with humoral immunodeficiency, these organisms occasionally develop prolonged, severe extraintestinal infection such as bacteremia, osteomyelitis, and arthritis (1, 3-5). We report a case of bacteremia, endocarditis, and osteomyelitis with *C. coli* in a patient with X-linked agammaglobulinemia. Bacteremia relapsed repeatedly in spite of treatment with combined intravenous antibiotics. *Campylobacter* was never isolated from stool cultures, but observed in the biopsy specimens from intestinal mucosa, suggesting the intestinal carriage. The addition of oral kamamycin with intravenous antibiotics was successful in eradicating *C. coli* from the blood, the bone and the intestine.

Case Report

A 33-year-old man with X-linked agammaglobulinemia was admitted to our hospital because of sustained fever and cellulitis of the left leg. The patient had history of recurrent diarrhea, upper respiratory infection and otitis media, and had been receiving immunoglobulin replacement therapy every two weeks. He had no history of overseas travel. He did not remember having raw meat such as chicken or coming into contact with animals in the past year. At the outpatient visits, oral cefcapene pivoxil and then intravenous ceftriaxone were administered, but the fever and cellulitis did not improve.

On admission, his temperature was 39.5℃, and his left leg was swollen. A grade 1/6 holosystolic cardiac murmur was heard. The abdomen was normal. The white blood cell count was 2,700/mm^3, the C reactive protein level was 1.09 mg/dL. The serum IgG was 893 mg/dL, but neither IgA nor IgM was detectable. Meropenem and then intravenous ciprofloxacin were administered for ten days. Seven days after discontinuation of the intravenous antibiotics, cel-

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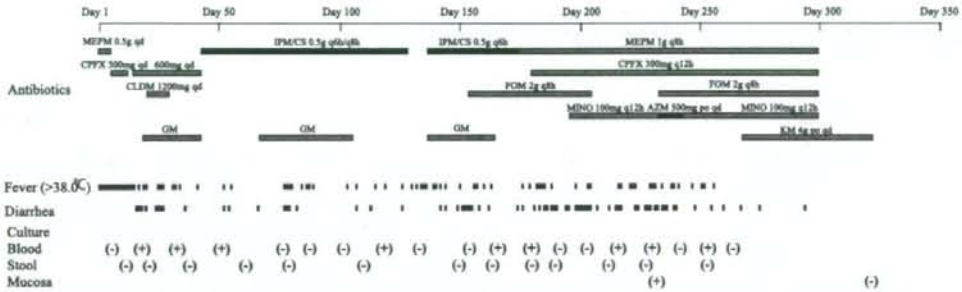

Figure 1. Clinical course of the patient. Antibiotics are shown in the upper panel. Fever and diarrhea are shown in the middle panel. Cultures from blood, stool and the intestinal mucosa are shown in the lower panel. MEPM: meropenem, IPM/CS: imipenem/cilastatin sodium, CPFX: ciprofloxacin, CLDM: clindamycin, FOM: fosfomycin, MINO, minocycline, AZM: azithromycin, GM: gentamicin, KM: kanamycin.

lulitis of his left leg worsened again. Blood culture was positive for *Campylobacter* species, which was determined as *C. coli* by a polymerase chain reaction assay. Stool cultures obtained repeatedly during the hospitalization did not yield *C. coli* (Fig. 1).

Intravenous administration of ciprofloxacin and gentamicin was started. Blood cultures continued to be positive for *C. coli*. A transesophageal echocardiography showed vegetation on the mitral valve, which suggested infective endocarditis. A colon fiberscope revealed mild inflammation. Antibiotics were switched to imipenem/cilastatin, and one week after the switch, blood culture turned negative. But four days after discontinuation of the 8-week antibiotic therapy, he became febrile again, and blood culture turned positive for *C. coli*. Intravenous imipenem/cilastatin and gentamicin were restarted. A magnetic resonance imaging study of the lower extremities revealed osteomyelitis of the left leg. Fosfomycin, which was susceptible in MIC test, was added to imipenem/cilastatin and gentamicin (Table 1). Gentamicin was discontinued because of renal tubular injury. Failing to eradicate *C. coli* from the blood, imipenem/cilastatin was switched to meropenem, ciprofloxacin and minocycline according to the susceptibility tests, and oral azithromycin, were sequentially added. In spite of disappearance of vegetation on the mitral valve in a transesophageal echocardiography and resolution of osteomyelitis of the left leg, bacteremia with *C. coli* continued (Fig. 1). During the combined antibiotic therapy, diarrhea and abdominal fullness became more prominent, although toxin A of *Clostridium difficile* was not detected from the stool. A colon fiberscope was performed again. The ascending colon was edematous, and the terminal ileum and the hepatic flexure were erosive. Although bacteria could not be detected histologically, all cultures of biopsy specimens sampled from the mucosa of the colon and the ileum yielded *Campylobacter* spp., suggesting intestinal carriage and reservoir of recurring infection.

Oral kanamycin was added to the intravenous antibiotics (Fig. 1). The following day his fever was resolved, and the abdominal symptoms improved. Blood cultures remained negative and intravenous antibiotics were discontinued six weeks after blood culture turned negative. A colon fiberscope was performed again, and oral kanamycin was discontinued after negativity of cultures of biopsy specimens from intestinal mucosa was confirmed. The total duration of oral kanamycin was ten weeks. The patient has been well for about eight months without relapse of bacteremia and diarrhea.

Table 1. The Minimum Inhibitory Concentrations to Antimicrobial Agents against *Campylobacter Coli* Isolated from the Blood of Our Patient

Antimicrobial agents	MIC (μg/mL)
Ampicillin	16.0
Sulbactam/ampicillin	32.0
Cefotaxime	>128.0
Cefpirome	>32.0
Aztreonam	>64.0
Imipenem/cilastatin	<0.5
Meropenem	<0.5
Gentamicin	<4.0
Erythromycin	8.0
Clarythromycin	8.0
Minocycline	16.0
Ciprofloxacin	8.0
Fosfomycin	<8.0

MIC: minimum inhibitory concentration

Discussion

Campylobacter coli is one of the most common *Campylobacter* species associated with diarrhea illness other than *C.*

jejuni, and these two species produce clinically indistinguishable infections (6). *C. coli* are present not only in food animals such as poultry, cattle, sheep, pigs, but also in domestic pets. Since *Campylobacter* species other than *C. jejuni* are difficult to identify with phenotypic testing, tests for detection of species-specific sequences via PCR have been developed (7). Bacteremia caused by *Campylobacter* species is uncommon and usually resolves spontaneously in immunologically normal hosts (2). In compromised hosts however, prolonged, severe and recurrent *Campylobacter* bacteremia and other extraintestinal infections may occur (2-4). In such patients, intestinal tissue invasion of *Campylobacter* spp. may be one of the pathogenetic mechanisms (2, 5). In normal subjects with *C. jejuni/coli* enterocolitis, serum IgA, IgM, and IgG antibodies to *C. jejuni/coli* rise rapidly after infection, and IgA antibodies in intestinal secretions also increase (8, 9). In patients with hypogammaglobulinemia or infected with human immunodeficiency virus (HIV), an impaired antibody response to *C. jejuni/coli* infection including bacteremia has been noted (10, 11).

There have been eleven case reports of X-linked agammaglobulinemia with *Campylobacter* bacteremia (12-19). Among them six cases had stool cultures positive for *Campylobacter* species and the other five cases had no description about stool cultures. Furthermore, among the six positive stool culture cases, five cases had no gastrointestinal symptoms such as abdominal pain or diarrhea. We speculated that intestinal carriage of *Campylobacter* with or without gastrointestinal symptoms might be a risk factor of recurrent bacteremia in immunocompromised patients. Stool cultures had sensitivity of 40% for *Campylobacter* bacteremia (1). In the present patient, stool cultures were consistently negative for *C. coli*, but cultures of biopsy specimens from intestinal mucosa were positive for *C. coli*. Furthermore in our case, infective endocarditis and osteomyelitis were clinically resolved in spite of persistent bacteremia. We speculated that the intestinal tract would be a reservoir for *C. coli*. The mechanism of intestinal carriage of *Campylobacter* in immunocompromised patients remained unclarified, but it has been supposed that failure of humoral immune response in these patients might permit colonization of *Campylobacter* in the epithelium and lamina propria and induction of tissue damage (20, 21). A study on *Campylobacter* infections in 38 HIV-infected patients reported that in two patients with diarrhea, the cultures were positive only in the blood, and that in one patient a culture of biopsy specimen from intestinal mucosa yielded *C. jejuni* after disappearance in stool culture (22). We consider that in compromised patients, who are suspected to be infected with *Campylobacter* but have negative stool cultures for bacteria, culture of intestinal mucosa might be useful for diagnosing the intestinal carriage.

Macrolides and fluoroquinolones are antibiotic agents frequently used to treat *Campylobacter* infection (2). However, in *C. coli* bacteremia in compromised patients, treatment with these drugs might fail, and combined therapy of intravenous antibiotics such as carbapenems and aminoglycosides has been used (18). There have been three reports in which administration of oral antibiotics in addition to intravenous antibiotics were effective in eradicating *C. coli* from the intestine (12, 15, 18). In the present case, oral antibiotics other than azithromycin had not been tried until isolation of *C. coli* from the culture of intestinal mucosa, because the intestinal carriage had not been considered from mild nonspecific inflammatory findings in the first colonoscope and repeated negative stool culture. Intestinal biopsy was not performed in the previous reports, and this is the first case in which culture of intestinal mucosa was used for diagnosing the intestinal carriage with *C.coli* and confirming the eradication by the treatment with oral antibiotics. Oral kanamycin was selected in our case because the isolated *Campylobacter* strain was susceptible to gentamicin. Since aminoglycosides including kanamycin have poor bioavailability, we considered that oral administration of kanamycin could not reach a sufficiently high serum concentration to treat endocarditis or osteomyelitis, but the administration could reach a sufficient concentration in the intestinal mucosa for eradicating *Campylobacter* spp. from the intestine. We suggested that in a case of bacteremia and intestinal carriage with *Campylobacter* which was refractory to intravenous antibiotics, oral aminoglycoside therapy combined with intravenous antibiotics could be effective.

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BMC Infectious Diseases

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Research article

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Acquisition of methicillin-resistant *Staphylococcus aureus* after living donor liver transplantation: a retrospective cohort study

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Abstract

Background: The incidence and risk factors of methicillin-resistant *Staphylococcus aureus* (MRSA) acquisition after living donor liver transplantation (LDLT) are unclear. The aim of the present study was to assess the incidence and to analyze the risk factors for the acquisition of MRSA after LDLT in adults by multivariate analysis.

Methods: We retrospectively reviewed the data from 158 adult patients that underwent LDLT at the Tokyo University Hospital. The microbiologic and medical records of the patients from admission to 3 months after LDLT were reviewed. Uni- and multivariate analyses were performed to identify the risk factors for postoperative acquisition of MRSA.

Results: Postoperative MRSA acquisition was detected in 35 of 158 patients by median postoperative day 18. Age (>= 60 y) and perioperative dialysis and/or apheresis predicted postoperative MRSA acquisition by multivariate analysis. In contrast, postoperative use of fluoroquinolone was negatively associated with acquisition of MRSA.

Conclusion: MRSA arose early after LDLT in adults with a high incidence (35 of 158 patients). Surveillance culture should be checked periodically after LDLT to identify and prevent the transmission of MRSA.

Background

Methicillin-resistant *Staphylococcus aureus* (MRSA) infection frequently complicates the postoperative course of deceased donor liver transplantation (DDLT) recipients [1-5]. In some centers, 91% (45 of 49 isolates) of all *Staphylococcus aureus* infections after DDLT are caused by MRSA [2].

Preoperative MRSA carriage is associated with an increased risk of MRSA infection after DDLT [1,3-5]. Positive MRSA culture in postoperative as well as in preoperative surveillance is important because the finding of MRSA colonization in a patient during hospitalization increases the risk of MRSA infection [6]. In one prospective study [6], the relative risk for developing MRSA infec-

tion in patients who had MRSA colonization was higher than that in patients who were not colonized with *Staphylococcus aureus*. In this particular study, 12 of 394 patients had MRSA colonization during hospitalization, and 4 of 12 (25%) later developed MRSA infection.

Few studies have focused on the factors associated with the acquisition of MRSA following liver transplantation. In one prospective study [7], the use of a urinary catheter for a prolonged period, postoperative bleeding at the surgical site, and preoperative use of fluoroquinolones independently increased the risk of MRSA colonization after DDLT. MRSA in cases of living donor liver transplantation (LDLT), in which operations are performed in a more scheduled manner, is not well documented.

The aim of the present study was to study the factors associated with the acquisition of MRSA after LDLT in adults assessed by surveillance cultures obtained from multiple sites, including nares, and to analyze the risk factors by multivariate analysis.

Methods

Patients

We retrospectively reviewed the data from 171 patients that underwent LDLT at the University of Tokyo Hospital, a 1150-bed teaching hospital, between August 2001 and November 2004. Of 171 patients, 13 were colonized with MRSA preoperatively and were excluded from the study. The median patient age was 51 years (range, 19–67). The indications for LDLT in these patients included hepatitis C (n = 53), hepatitis B (n = 24), primary biliary cirrhosis (n = 24), fulminant hepatitis (n = 18), biliary atresia (n = 8), autoimmune hepatitis (n = 7), primary sclerosing cholangitis (n = 5), metabolic disease (n = 5), alcoholic cirrhosis (n = 4), cryptogenic cirrhosis (n = 2), and others (n = 8). Of the 158 patients, 68 had hepatocellular carcinoma. The median Child-Pugh score and model for end stage liver diseases (MELD) score of those patients was 10 (range, 5–14) and 13 (range, -3 to 48), respectively. The microbiologic and medical records of the patients from admission to 3 months after LDLT were reviewed. The present study was approved by The University of Tokyo Ethical Committee. The data used for the study are publicly available.

Donor selection

Donors were selected from the patients' relatives. Age, blood type, graft size, and liver function were also taken into consideration. ABO blood groups were required to be identical to or compatible with that of the recipients. The graft type was determined according to the ratio of the estimated graft volume to the recipient's standard liver volume ratio [8,9]. Our surgical technique for recipient and donor surgery is described elsewhere [10]. Donors were not routinely screened for *Staphylococcus aureus* perioperatively.

Perioperative management

Antimicrobial prophylaxis consisted of intravenous cefotaxime (1.0 g just before surgery, followed by 1.0 g every 6 hours intraoperatively and thereafter), ampicillin/sulbactam (1.0 g just before surgery, followed by 1.5 g every 12 hours intraoperatively and thereafter), and gentamicin, 60 mg every 12 hours after surgery) for 5 days.

To prevent fungal infection, fluconazole (200 mg every 24 hours) was administered intravenously for 7 days after surgery. All patients received the same immunosuppressive regimens using tacrolimus (Prograf, Astellas Pharmaceutical Corporation, Tokyo, Japan) and methylprednisolone (Solu-Medrol, Pfizer Inc., New York, NY). The details of the regimen are reported elsewhere [11].

Definition of MRSA colonization

All the patients were screened preoperatively for *Staphylococcus aureus* on admission for LDLT. Follow-up specimens were collected twice a week during the first month after LDLT, and thereafter once a week during the hospital stay. Routine surveillance specimens consisted of swabs of the anterior nares, pharynx, sputum, urine, and stool. In addition, swabs of wound or skin lesions, bile, and discharge from the abdominal cavity were collected postoperatively. Blood samples, collected percutaneously, and a segment of a removed intra-vascular catheter were also submitted when infection was suspected as the followings: fever (> 38°C), chills, or hypotension. Other clinical samples were added in patients with suspected infection according to the discretion of the attending physician.

Specimens were plated onto mannitol-salt agar or sheep blood agar. *Staphylococcus aureus* was identified using standard microbiologic methods.

Methicillin resistance was determined using a disk diffusion test performed on Mueller-Hinton agar after incubation for 24 to 48 hours at 30°C. By the microdilution method, strains with an oxacillin minimum inhibitory concentration value of at least 4 μg/ml were defined as MRSA [12]. Patients colonized with *Staphylococcus aureus* at any site, and at any time during the hospital stay, were considered carriers.

Definition of MRSA infection

Nosocomial infections were defined according to the reports from the Centers for Disease Control and Prevention in 1988 and in 1992, as described elsewhere [13,14]. When MRSA was isolated from culture samples in the